ORIGINAL ARTICLE

NS1 Dengue Antigen among Blood Donors in Two Blood Collection Centers in North Malaysia

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ABSTRACT

Introduction: Dengue virus is one of the emerging agents that can be transmitted via blood transfusion from infected blood donors to recipients. In Malaysia, the increase in dengue infection may contribute to the existence of asymptomatic blood donors and increase the risk of blood supply contaminated with this virus. The aims of this study were to investigate the prevalence of NS1 dengue antigen among blood donors and to ascertain the demographic data of blood donors in Penang and and Perak. **Methods:** A total of 374 voluntary blood donors were recruited from two blood donation campaigns organised by Hospital Pulau Pinang, Penang and Hospital Raja Permaisuri Bainun, Ipoh, Perak from April to May 2016. From each centre, 187 voluntary blood donors were enrolled, blood was collected and Dengue NS1 Ag was screened on all the samples using Platelia dengue antigen test kit from Bio-Rad Laboratories, France. **Results:** All 374 samples were found to be negative for the Dengue NS1 antigen. Demographic data of these blood donors showed that the most common blood group was O Rh positive, men donated more than women and Chinese blood donors were the biggest group of donors. **Conclusion:** Even though dengue is endemic in Malaysia, none of the blood donors was screened positive for dengue NS1 antigen in the areas studied. This indicates that none of the blood donor at the time of donation was in viraemia stage. The established donor screening program ensures that the dengue transmission through transfusion is minimal in the areas studied.

Keywords: NS1 Dengue Antigen, Blood donors, North Malaysia

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INTRODUCTION

Dengue is a vector-borne disease that leads to health problems worldwide. Dengue virus consists of single stranded RNA genomes surrounded by an envelope. The genome corresponds to a range of nucleotides, which encode 3411 amino acids. The entire genome contains seven proteins, with three structural proteins, membrane protein, core and envelope protein, besides seven nonstructural proteins, including NS1, NS2a, NS2b, NS3, NS4a, NS4b and NS5 (1). The presence of NS1 protein in the process of viral replication is not known, but it gives the impression in terms of virus infection and the development of a disease or morbid condition (2).

Overall, 100 million of dengue cases are estimated per year, with roughly 500 000 severe dengue patients requiring continuous treatment in hospitals (3). Dengue infection has been prevalent in many countries including Africa, the Americas, the eastern Mediterranean, Southeast Asia and the Western Pacific. In 2011, there is an increase in dengue cases documented in the United States and Brazil, with 1.1 million cases and 764 305 cases of severe dengue (4). A rapid spread of dengue, very likely could extend and alter antigenic properties of the virus, including new serotypes and genetic make-up with a larger outbreaks possibility (5).Treatment and deaths caused by dengue among children in Asia has exceeded malaria in the mid-70s (6).

After an epidemic of dengue infections in 1973, many dengue cases were reported in Malaysia (7). A high and persistent incident of dengue cases were documented in the presence of infection by all serotypes (8). In the early 1990s, approximately 5000 cases were documented each year. A dramatically increase in pattern of dengue cases were reported in 1999 to 2007, with 44.3 cases to 181 cases per 100,000 population (7). Continuous increase in dengue cases were documented in the country, since November 2015 until the first week of 2016. Overall,

there were 12,138 cases (11.2%) reported in 2015 compared to 108,698 cases in 2014. Statistics from the Ministry of Health, Malaysia showed an increase in dengue cases from fifth until eleventh June 2016. The numbers of dengue cases raised to 1585 cases compared to 1272 cases in the previous week. Perak showed an increase of 21 cases and Penang showed an increase of 19 cases. In the trend of continuous increase in dengue cases, the possibility of the existence of asymptomatic blood donors is very high for both states (9).

An asymptomatic blood donor who is already contaminated with the dengue virus can cause a symptomatic infection when blood is transfused to a recipient. During epidemics of dengue in Puerto Rico, Brazil, dengue screening was performed on donated bloods (10). Results through nucleic acid testing (NAT), found 12 donors out of 16521 donors were positive for dengue (11). Dengue infection through blood transfusion has also been reported in Singapore. Both recipients of packed cells and plasma products (fresh frozen plasma), developed fever after transfusion. Screening tests that had been carried out to the recipient using PCR, gave positive results for dengue antigen (12). A dengue screening program conducted among 329 volunteer donors in an area with a high number of dengue cases in Thailand, found 29 donors positive for dengue Ig M while 2 donors were positive for dengue RNA (13). In Indonesia, a study of dengue was carried out on 785 volunteer donors. Of these, eight (8) dengue infected asymptomatic donors have been identified (14).

Asymptomatic blood donors involve apparently healthy donors during the blood donation process; without any symptoms of infection, although they may have been infected with dengue virus. Dengue screening tests are not carried out in the blood collection centres because the prevalence of NS1 dengue antigen among blood donors and the risk of infection through blood donor are not well established in Malaysia. Awareness of dengue infection through blood transfusion and blood components are still low and most of the previous studies involved patients. There is no realistic statistical data available for the prevalence of NS1 dengue antigen among blood donors in Malaysia to establish dengue virus antigen occurrence. Therefore, this research was conducted to in two (2) blood collection centers in the Northern part of Malaysia with the hope to establish an awareness of dengue as an emerging agent and thus reduces the risk of occurrence of blood product contamination.

MATERIALS AND METHODS

Donor Recruitment

Inclusion and exclusion criteria for blood donors involved with this study were donors who meet the criteria set by the Ministry of Health (MOH) Transfusion Service Practice Guidelines for clinical and laboratory personnel (15).

Sample size calculation

This study was conducted with 374 experimental subjects, with 10% drop out. The power of study is 0.8 (80% power = the confidence interval) and the type 1 error probability associated with this test of this null hypothesis is 0.05. The sample size was calculated by using Power and Sample software, which was developed by Duppont and Plummer, 1997 (16) and based on Dichotomous formula.

Sample collection and Storage

The study subjects were voluntary non-remunerated blood donors attending to two mobile blood donation campaign Hospital Pulau Pinang and Hospital Raja Permaisuri Bainun, Perak. One hundred and eighty-seven (187) donors volunteered to participate from each donation campaign. Informed consent was obtained from all blood donors. At least three milliliters (3 mL) of whole blood was obtained from the sampling pouch of the participant's donor blood bag into a vacutainer tube containing ethylene diamine tetra acetic acid (EDTA) as anticoagulant. The samples in EDTA tubes were centrifuge and plasma were removed. The plasma were stored at 2°C – 8°C in a refrigerator at Regenerative Lab, Advanced Medical and Dental Institute (AMDI), Universiti Sains Malaysia (USM), Penang.

Sample processing

Dengue NS1 antigen test was performed using the Platelia Dengue Ns1 Ag (Bio-Rad Laboratories, France) within 24 hours after sample collection, according to the procedures established by the manufacturer at Regenerative Lab, AMDI, USM, Penang. The entire samples were discarded according to the standard operation procedure within 24 hours after the test was performed and validated.

There were several factors to consider in assessing the risk of transfusion-transmitted dengue in the samples for example lipaemic samples, however this factor has been excluded.

Data collection

Personal information such as blood donor names, phone numbers, home addresses and identity card numbers were not included in the data collection process. Data collection form was used to collect donor blood group, barcode number of the blood bag, age, sex and race. All information and data collected from blood donors is confidential.

Statistical analysis

Descriptive data analysis was calculated using IBM SPSS statistical package version 22.

Ethics

Ethical approval for this study was received from Ministry

Of Health Research and Ethics Committee (MREC) with registration ID, NMRR-15-1822-28165 (Ref. No. (9) KKM/NIHSEC/P16-85) and USM Ethics Committee with approval number, USM/JEPeM/16010003. There were no conflicts of interest in any form of this research during donor recruitment and any parts of the study.

RESULTS

Screening for NS1 dengue antigen

Screening result for each sample was expressed by sample ratio by dividing the optical density of the sample with the cut-off value. Optical density (OD) reading will be higher for the sample with a higher reactivity. In average, the cut-off value in screening all samples was more than 0.2 (> 0.2). Ratio for all the donor samples was below than 0.50 (<0.50), in which the results interpretation and recommendations were negative for Dengue NS1 antigen. Interpretation and recommendations were based on manufacturer protocols.

Demographic data of blood donors in Penang and Perak

There is a statistically significant difference between age of donors with gender (p<0.001) and age with ethnicity (p=0.001). However, there is no statistically significant difference between age of donors with blood group (p=0.694). Older donors were male, Chinese ethnicity and blood group AB (Table I).

Table I: Association between age of donor with the demographic characteristics

Variables	Age in years (mean, SD)	*P* value
Gender		
Male	38 (11.1)	<0.001 [a]
Female	33 (10.9)	
Ethnicity		
Malay	29 (10.7)	0.001 [b]
Chinese	37 (11.2)	
Indian	33 (9.6)	
Blood Group		
A	37 (10.3)	0.694 [b]
AB	38 (13.7)	
B	36 (11.9)	
O	35 (11.6)	

[a] Independent t-test
[b] One-way Anova test

The minimum donor age was 17 years old, while the maximum age of the donor was 63 years old. On the whole, the mean age for blood donors was 36.6 (Table II). All blood donors in Perak and Penang consists of the three majority races in Malaysia, which are Malays, Chinese and Indians. The number of Chinese blood donors were the highest with 338 donors. Chinese blood donors include 90.4% of the total number of blood donors, followed by Malay (7.2%) and Indian (2.4%). A male predominance was observed amongst the 374 blood donors recruited from both Perak and Penang (Table II). The number of male donors volunteered totaled 242 accounting 64.7% of the total. As expected, donors with O positive blood group was the predominant representing 42% (157) of the total participants while B positive donors (111) accounts for the second commonest representing 29.7% of the total. The third most common blood type amongst the study sample was A positive (88) accounting for 23.5%. The rest is AB positive represented by 18 donors making up to 4.8% (Table II).

Table II: Demography of the blood donors from both collection centers

Variables	Perak (No. of donor)	Penang (No. of donor)
Age		
17-27	33	85
28-38	38	52
39-49	60	42
50-59	54	8
60-69	2	0
Gender		
Male	145	126
Female	42	61
Ethnicity		
Malay	3	24
Chinese	181	157
Indian	3	6
Blood group		
A	45	43
B	10	8
AB	50	59
O	82	77

Total of donors for each group 187

DISCUSSION

In this study, dengue NS1 antigen screening was performed on all blood samples collected from two blood donation centers between April to May 2016 in Perak and Penang. The non-structural protein 1 (NS1) antigen in the form of immune complexes are demonstrated in high concentrations in patients with both primary and secondary dengue infections up to nine days after the onset of illness. This immune complex, Dengue NS1 antigen, was screened using Enzyme Linked Immunosorbent Assay (ELISA) methods. Dengue NS1 antigen test was chosen for screening because of the effectiveness in detecting early infections and asymptomatic donors.

Interestingly, the screening of dengue NS1 antigen for

blood donor's samples in this study was all negative. This suggests that the stringent donor recruitment process used in Malaysia is highly efficient in screening out the asymptomatic and viraemic dengue and the risk of transfusion transmitted dengue is significantly minimal despite the endemic dengue in Malaysia. Extensive literature search did not find any transfusion-associated dengue case in Malaysia. Prospective donors recovered from dengue are deferred for 6 months after full recovery. This period of deferral can reduce the transmission of dengue via transfusion, by identify or recruiting only truly healthy blood donors.

Prevalence rate of certain infection plays an important role to indicate either a disease or infection should be included in the national donor screening protocol. In this study, dengue NS1 antigen was not detected in the sample of blood donors in Perak and Penang, ensuring the viraemic donation is extremely low. Prevalence rates obtained through this study are similar to the findings of dengue viraemia among blood donors carried out in Northern India. Dengue NS1 antigen screening was done on 1709 blood donors and all results were negative (17).

Qualities of samples used in the screening of dengue NS1 highly contribute to the results of screening. Although samples containing 100 mg/L bilirubin and lipaemic samples containing the equivalent of 36 g/L triolein (triglyceride) do not affects the results, but presence of albumin at 90 g/L or haemolysed samples containing 10 mg/ml of hemoglobin can potentially increase ratio of negative samples. Since in this study, albumin level in the samples was not measured, negative results due to the presence of albumin could not be established (18).

Moreover, incorrect volume reconstitutions of reagents used for screening purpose could invalid the results. Some of the reagents used in the screening procedure are not a "ready to use products". Reagents reconstitution was performed manually for washing solutions, conjugate and diluent. Contamination of the stopping solution and contamination of the development by oxidizing agents such as bleach and ions could cause non-reproducible reactions (18).

Furthermore, technical skills while conducting screening tests also play an important role in determining reliable screening results. After the incubation period in the assay procedure, microplate was required to be washed 6 times with washing solution manually. Washing the microplate is a critical step in the procedure, because incorrect washings may lead to inaccurate results. Inadequate microplate washings often results to in-validated and non-reproducible reactions (18).

Sensitivity and specificity of screening assay could indicate the detection level of a specific kits used for screening NS1 dengue antigen. For screening purpose, it is highly recommended to use a more sensitive assay to detect dengue antigen. The sensitivity of Platelia Dengue NS1 antigen test kit ranges from 87.1 % to 100 %, related to a specific virus serotype. Dengue serotype 2 was highly dominant in Malaysia (8). Sensitivity of Platelia Dengue NS1 antigen test is only 87.1 % towards dengue serotypes 2 and this could lead to undetectable level of dengue antigen among blood donors.

Viral load in a blood donors sample, indirectly contribute to the detection of dengue NS1 antigen. Screening assays that are less sensitive could not detect blood donors sample with low viral load. Humans infected with dengue virus infection typically have a virus titer of 10 5-10 9 copies / mL (17). Therefore, asymptomatic blood donors with low viral load most likely will not be detected by the screening method employed in this study. Apart from that, presence of antibodies in the sample may also indirectly interfere with the screening of dengue virus antigen (3).

Although in principle, all serotypes of dengue virus can be screened throughout the year, the peak season of dengue infection should be given attention. Highest numbers of dengue cases are generally reported during the rainy season of each year from January to March and between June and November (19). Since the screening for dengue NS1 antigen in this study was conducted between April to May 2016, the risk may have been underestimated.

In addition, sample quality also plays an important role in screening for dengue NS1 antigen. Haemolysed samples may contribute to invalid results. Invalid screening results include false positive and false negative results (18). Moreover, samples with fibrin or contaminated with bacteria can also contribute to the quality of screening results. Technical mistakes and failure to follow the manufacturer's instruction may also contribute to false positive or false negative results.

Most blood donors from the age of 20 years up to 26 years old were students from universities and colleges nearby in Penang. In Perak, blood donors in the range of 17 to 26 year old were members of religious associations. This data could be used for blood donor management specifically for donor recruitment and planning location for a blood donation campaign for the target age group. Blood donors from Perak and Penang were inclusive of all four major blood groups, including, A, B, AB and O. All blood groups were Rhesus (D) positive.

Through data analysis conducted during the study period, the number of male blood donors was more than female blood donors. The tendency for men to donate blood more than women were due to the nature of man. In addition, female blood donors have lower intention to donate blood (20). This data could be used to plan an awareness of blood donation among women. Through the analysis of demographic data collected during blood

donation camps in Perak and Penang between April and May 2016, found that all the major races in Malaysia were represented in this study. The numbers of Chinese blood donors was the highest in Perak and Penang. This data could be used for donor recruitment with a purpose of looking for a specific blood group or specific genotype and phenotype bloods within ethnic in Malaysia. In the Rh system, the prevalent Rh phenotype amongst Chinese are R2R2 (DcE / DcE), R1R1 (DCe / DCe) in Malays and rr (cc / cc) in Indians (21).

CONCLUSION

Even though dengue is endemic in Malaysia, none of the blood donors in this study was screened positive for dengue NS1 antigen in the areas studied. This translates that the established donor screening program ensures that the dengue transmission through transfusion is minimal at least in these studied areas. Although NS1 dengue antigen were not detected among the blood donors, further studies are needed to be done in different dengue prone districts using more sensitive molecular techniques with a larger sample size. Further research should be conducted, to evaluate, educate and describe the importance of dengue antigen screening in donor laboratory screening and the risks of transmission of dengue through blood transfusion. Further study should also look at screening methods that are most suitable to be performed to screen NS1 dengue antigen, either using a combination of antigen - antibody testing or NAT. Demographic data collected through this study is expected to be useful in future planning of blood donation, blood donor recruitment, blood stock management according to blood group and blood inventory management according to the needs and the current target.

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